

**Int. Appl. No. : PCT/BE2003/000147**  
**Int. Filing Date : September 3, 2003**

## **AMENDMENTS TO THE SPECIFICATION**

**Please add the following paragraph immediately after the Title of the Invention:**

### **Related Applications**

This Application is the U.S. National Phase under 35 U.S.C. 371 of International Application No.: PCT/BE2003/000147, filed September 3, 2003 designating the U.S. and published in English on March 18, 2004 as WO 2004/022745, which claims the benefit of priority of U.S. Provisional Patent Application No. 60/408,482, filed September 3, 2002, the entire disclosure of which is hereby expressly incorporated by reference.

**Please replace paragraph [0005] on page 2 of the Specification with the following paragraph:**

A first aim of the invention concerns methods and tools which provide a solution to the above-mentioned problems, in particular methods and tools which allow a molecular biologist to insert and/or remove a genetic element, or to obtain a modification in the ~~lecture~~ reading orientation of said genetic element (inversion) in a nucleotide sequence, either *in vitro* or *in vivo*.

**Please replace paragraph [0010] on page 3 with the following paragraph:**

In the method according to the invention, said foreign nucleotide elements are advantageously linked (at its 3' or 5' or both ends) to one or more promoter/operator nucleotide sequences, such as, but not limited to, constitutive promoters allowing the expression of a target nucleotide sequence incorporated in the nucleic acid construct according to the invention, when they are disposed according to the suitable and requested ~~lecture~~ reading orientation.

**Please replace paragraph [0013] on page 4-5 with the following paragraph:**

The elements used in the method of the invention are specific cells and a genetic preferably integrated in a vector or a chromosome of a cell comprised of either:

- a promoter/ activator sequence 11 disposed upstream of a first and a second nucleotide sequence (1, 2) encoding two different toxic molecules (such as a poison 1 and a poison 2) (figure 2, left), or

- a first promoter/activator sequence 11 disposed upstream of a first nucleotide sequence 1 encoding a toxic molecule (such as a poison 1) and, disposed in the opposite ~~lecture~~ reading direction of the first promoter/activator sequence 11, a second promoter/activator sequence 12 disposed upstream of a second nucleotide sequence encoding an antidote 2' to a second toxic molecule (such as poison 2) (figure 3, left), or

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-a promoter/activator sequence 11 disposed upstream of a first and a second nucleotide sequence (1,2') encoding, respectively, a first toxic molecule (such as poison 1) and an antidote to a second toxic molecule (such as poison 2) different from said first toxic molecule (figure 4, left).

-The terms "a nucleotide sequence encoding a toxic molecule or an antidote to a toxic molecule" also include sequences comprising multiple coding portions encoding several identical toxic molecules.

**Please replace paragraph [0018] on page 6-7 with the following paragraph:**

This reversible cloning and selection method is also suitable for obtaining an inversion of an integrated genetic element. A specific example is described in details hereafter, in reference to the figure 5. Indeed, the orientation of a sequence of interest can be reversed through the method of the invention (preferably following the insertion step of figure 4) or through a direct insertion of the target sequence between two different antidote sequences (1', 2'). Said genetic element (target sequence) associated to a promoter/operator (either at its 3' or 5' end), is initially integrated between two nucleotide sequences (1', 2') encoding respectively two different antidotes to two different toxic molecules 1 and 2. Said two nucleotide sequences (1', 2') encoding the two different antidotes are disposed in opposite ~~lecture~~ reading orientations (disposed upstream and downstream the target nucleotide sequence in opposite divergent ~~lecture~~ reading orientation). This construct allows to select for the recombination event(s) which will cause the target nucleotide sequence of interest and its associated promoter to have either the same orientation as the nucleotide sequence 1' encoding the first antidote to the first toxic molecule (selection done in a strain both sensitive to and producing poison 1) or the same orientation as the nucleotide sequence 2' encoding the second antidote to the second toxic molecule (selection done in a strain both sensitive to and producing poison 2). (see WO 02/066657 incorporated herein by reference)

**Please add an Abstract submitted herewith as the last page of the Specification.**